IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	*Application Number*	10/806,038
	Filing Date	March 22, 2004
	First Named Inventor	SALLY MACKENZIE
	Group Art Unit	1638
	Examiner Name	A. Kubelik
	Attorney Docket Number	1231-218
Title of the Invention: **IMPLEMENTATION OF A MITOCHONDRIAL MUTATOR**		

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner of Patents
P. O. Box 1450
Alexandria VA 22313-1450

Sir:

I, Sally Mackenzie, of 920 N. 88th Street, Lincoln, Nebraska 68505, hereby declare that: I am employed by the University of Nebraska as a professor. Since 1982, I have been involved in scientific research on cytoplasmic genetics of plants.

I am the inventor of the invention described and claimed in the above-identified application and am familiar with the Advisory Action dated December 8, 2006.

I submit that mutation of the *MutS homolog 1* (*Msh1*) locus, previously designated *Chm* (*Chloroplast mutator*) on Chromosome 3 does not create a condition of cytoplasmic male sterility (CMS) in *Arabidopsis thaliana*.

Data from my laboratory include testing *msh1* mutants in ecotypes Columbia-0 (Col-0), Landsburg erecta (LER), Aa-0, Dijon G, Stockholm, Ws (Wassilewskija), Hodja, Shakdara, and Ang-0 to support the above statement. In addition, the above statement is also supported by data deduced from publications by G.P. Redei (Extra-chromosomal Mutability Determined by a Nuclear Gene Locus in Arabidopsis, *Mutation Research*, 18, 149-162, 1973), Martinez-Zapater et al. (Mutations at the Arabidopsis *CHM* Locus Promote Rearrangements of the Mitochondrial Genome, *Plant Cell*, 4, 889-899, 1992), and Sakamoto et al. (Altered mitochondrial gene

expression in a maternal distorted leaf mutant of Arabidopsis induced by chloroplast mutator, *Plant Cell*, 8:1377-90, 1996).

Cytoplasmic male sterility (CMS) is defined by the scientific community as a maternally inherited trait, mitochondrially conditioned, that influences the plant's ability to produce and/or shed viable pollen, with no detectable influence on female fertility and generally no other plant morphological abnormalities (Hanson MR, Bentolila S. Interactions of mitochondrial and nuclear genes that affect male gametophyte development, *Plant Cell* 16, Suppl:S154-69, 2004). The CMS trait is distinguished from physiological stress-induced forms of floral sterility by its uniformity of expression across the plant, inheritance pattern (100% male sterile F1 progeny follow pollination with maintainer pollen), and full female fertility. In our studies of Arabidopsis, and those reported by Redei, Martinez-Zapater et al., and Sakamoto et al, any evidence of male sterility was accompanied by varying degrees of female sterility, floral developmental irregularities, abnormal plant growth (aberrant leaf morphologies) and severe leaf and floral variegation (plastid dysfunction). In Martinez-Zapater, for example, the authors state "...completely chlorotic sectors lacking green subsectors grew much more slowly than variegated sectors and produced flowers that were male sterile" (pg. 890). These plants were able to produce selfed progeny from their normal flowers. In Sakamoto et al, the authors describe crosses to the *chm* mutant that result in a distorted leaf phenotype. "This mutant, maternal distorted leaf (MDL), grows very poorly and is distinguished by distorted rough leaves and aborted flowering organs" (see abstract), resulting in both male and female sterility directly associated with the dramatically altered plant morphology. In Redei (1973), the author states "The [male and/or female] sterility was especially high among the plants with distorted leaves; the white or very pale shoots also displayed much reduced fertility" (pg. 153).

This type of aberrant flower development, accompanied by male and female sterility, is associated with plastid dysfunction of the plant (not the CMS mutation), is most pervasive in regions of the plant that display variegation or abnormal leaf morphologies, and displays poor heritability. In my own laboratory, this condition is only observed in the first generation following introduction of the *msh1 (chm)* mutation, but disappears with loss of variegation in subsequent generations. CMS occurs in a regular pattern that affects all flowers of the plant,

shows clear and recurrent maternal inheritance, is not associated with female infertility, and is associated with mitochondrial genetic lesions, not plastid dysfunction. While many forms of male and/or female sterility can occur in plants, some genetically conferred and some conferred by physiological or environmental stress, cytoplasmic male sterility constitutes a well defined trait that is not to be confused with these. Therefore the mutation of the *MutS homolog 1* (*Msh1*) locus, previously designated *Chm* (*Chloroplast mutator*) on Chromosome 3 does not create a condition of cytoplasmic male sterility (CMS) in *Arabidopsis thaliana*.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: February 13, 2007

Sally Mackenzie

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